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Research Article

TOXICOLOGICAL EVALUATION OF INSECTICIDES ON *BRACON HEBETOR* (SAY, 1836) UNDER CONTROLLED LABORATORY CONDITIONS

[a]Dilbar Hussain, [b]Sabeen Asghar, [c]Khuram Zia, [b]Muhammad Asrar, [d]Saba Malik, [b]Usama Saleem

[a] *Entomological Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan.*
[b] *Department of Zoology, Faculty of Life Sciences, Government College University Faisalabad, Punjab, Pakistan.*
[c] *Department of ORIC, University of Agriculture, Faisalabad, Pakistan.*
[d] *Engro Fertilizers Limited, Pakistan.*

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ABSTRACT

Bracon hebetor (Say) is an eco-friendly and important biological control agent against numerous destructive insect pests worldwide. Its beneficial role is increasingly attracting the attention of growers seeking sustainable pest management solutions. However, some conventional and novel insecticides exhibit toxic effects on *B. hebetor*. The present study aimed to assess the toxicity of six commonly used synthetic insecticides, emamectin benzoate, acetamiprid, chlorpyrifos, imidacloprid, lufenuron, and flubendiamide, against the larval parasitoid *B. hebetor* under laboratory conditions (29 ± 1°C; 60 ± 5% RH). The experiment was conducted at the Toxicology Laboratory of the Entomological Research Institute, AARI, Faisalabad, Pakistan, using the vial residual method. Among the tested insecticides, chlorpyrifos was the most toxic to *B. hebetor*, with LC_{50} values of 1901.16, 249.79, 81.13, 44.85, and 34.99 mg/L at 3, 6, 12, 24, and 48 h post-exposure, respectively. Acetamiprid and imidacloprid exhibited moderate toxicity, with LC_{50} values of 31.75 mg/L and 223.95 mg/L at 48 h, respectively. In contrast, lufenuron showed lower toxicity (LC_{50} = 877.87 mg/L at 48 h), while emamectin benzoate and flubendiamide were the least toxic, with LC_{50} values of 580.28 mg/L and 2,325.36 mg/L, respectively. These findings indicate varying levels of toxicity among the tested insecticides, highlighting the need for careful selection of insecticides to minimize adverse effects on *B. hebetor*. To maintain ecological balance and promote biological pest control, insecticides with the lowest impact on *B. hebetor* should be used judiciously.

Corresponding Author: Muhammad Asrar
Email: asrar@gcuf.edu.pk
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INTRODUCTION

Bracon hebetor Say (Hymenoptera: Braconidae) is an ectoparasitoid known for its gregarious behavior. As a larval parasitoid, it primarily targets the larval stage of various lepidopteran species (Ahmed and Ahmad, 2006; Ebeid et al., 2017; Asadi et al., 2021). Being a cosmopolitan parasitoid, it belongs to a family that comprises more than 21,000 known species worldwide (Quicke, 2015; Chen and Achterberg, 2019; Afshari et al., 2020).

B. hebetor has gained attention as an effective biological control agent against various pests due to its short

lifespan and remarkable reproductive ability (Dweck et al., 2008). Furthermore, it has a broad host range and is considered a polyphagous parasitoid of several lepidopterans and stored grain pests (Desai et al., 2007; Shim et al., 2008).

The species has a short larval stage and a prolonged pupal stage. During the larval stage, it serves as a significant parasitoid, effectively targeting and controlling various agricultural insect pests, particularly moths of the families Noctuidae and Pyralidae (Mbata and Shapiro-Ilan, 2010). The average life cycle duration from egg to adult is approximately 11 days under optimal conditions. However, the life cycle can be influenced by unfavorable environmental factors. When relative humidity drops below 30%, the pupae enter diapause but resume development when conditions become favorable (Adams et al., 1969).

B. hebetor employs a strategy wherein it renders its host (*Galleria mellonella*) immobile by stinging and injecting paralytic venom (Adly and Marzouk, 2019). These hosts are usually in their final larval stage, a phase known as 'wandering'. After paralyzing the host, the female wasps lay a variable number of eggs either on or near the immobilized host, ensuring the successful multiplication and completion of the *B. hebetor* life cycle (Alam et al., 2016; Adly and Marzouk, 2019).

Synthetic insecticides are harmful to the environment and the natural enemies of insect pests. Several studies have reported that insecticides such as chlorpyrifos, profenofos, and lambda-cyhalothrin exhibit high toxicity to *B. hebetor*, whereas newer molecules like chlorantraniliprole and spinosad have relatively lower adverse effects (Ahmed and Ahmad, 2006; Muslim et al., 2018). Considering the potential negative impact on non-target beneficial insects, the judicious use of synthetic insecticides is crucial for the success of integrated pest management (IPM) programs. The objective of this study was to assess the toxicity of synthetic insecticides against *B. hebetor* and determine its average percent mortality. Furthermore, the study aimed to analyze the lethal and sub-lethal doses of the insecticides.

MATERIALS AND METHODS

The experiment was conducted at the Toxicology Laboratory of the Entomological Research Institute, Ayub Agricultural Research Institute (AARI), Faisalabad, Punjab, Pakistan. As the research focused on evaluating the toxicity of synthetic insecticides against *B. hebetor*, the host insect was reared on an artificial diet and maintained under controlled laboratory conditions.

Rearing of *B. hebetor*

Adult *B. hebetor* were collected from a maize field and brought to the laboratory for rearing. Fourth-instar larvae of the wax moth were carefully separated and placed onto paper cards measuring 5 × 7 inches. After 48 h, the larvae settled into the crevices of the paper cards and spun cocoons. These cards, containing the wax moth cocoons, were then provided to adult *B. hebetor* for subsequent parasitism and mass rearing.

Female parasitoids deposited their eggs on the host larvae, and these eggs typically hatched within 2-3 days. Upon hatching, the larvae emerged and began feeding on the host. After approximately 17-25 days, the larvae transformed into pupae, entering a non-feeding stage. Within 3-5 days, adult parasitoids emerged from the pupae. The rearing process was maintained under laboratory conditions at 29 ± 1°C, 65 ± 5% RH, and a 12:12 (light:dark) photoperiod.

Insecticides

The six synthetic insecticides listed in Table 1 were evaluated in the laboratory bioassay.

Bioassay

The vial residual method was used to evaluate the toxicity of six synthetic insecticides against *B. hebetor*. Serial dilutions of each insecticide were prepared. Whatman filter papers, cut to fit the vials, were dipped in the insecticide solution for 10 sec. The soaked filter papers were then removed using forceps and left to air dry for 15 min. Once dried, these filter paper strips were placed inside the vials to assess the insecticidal toxicity against *B. hebetor*. Five adult *B. hebetor* were introduced into each vial to measure the mortality rate of the parasitoid. The experiment was conducted in five replicates under controlled laboratory conditions at a temperature of 29 ± 1°C and a relative humidity of 65 ± 5%.

Data analysis

Mortality data were recorded at 3, 6, 12, 24, and 48 h. The normality of the data was assessed using the Shapiro-Wilk test. Probit statistical analysis was performed to determine LC_{50} values (Finney, 1971). One-way ANOVA, followed by Tukey's HSD test, was conducted using IBM SPSS software to compare treatment effects, with significance set at $p < 0.05$.

Table 1. Synthetic insecticides, their brand names, and recommended field doses.

Sr. No.	Common name	Brand name	Field recommended doses
1	Emamectin Benzoate	Proclaim 1.9EC	200ml
2	Chlorpyrifos	Lorsban 48EC	1000ml
3	Acetamiprid	Mospilone	125g
4	Imidacloprid	Confidor 200SL	250ml
5	Lufenuron	Match	200ml
6	Flubendiamide	Belt 480SC	50ml

RESULTS

Toxicity evaluation of insecticides against *B. hebetor*

Table 2 presents the LC_{50} values of six insecticides at different time intervals: 3, 6, 12, 24, and 48 h. After 3 h, the LC_{50} value of chlorpyrifos was 1901.16 mg L^{-1}, followed by acetamiprid (1397.09 mg L^{-1}), imidacloprid (16,770.81 mg L^{-1}), lufenuron (19,490.57 mg L^{-1}), flubendiamide (52,893.76 mg L^{-1}), and emamectin benzoate (54,089.84 mg L^{-1}). After 6 h, the LC_{50} value of chlorpyrifos decreased to 249.79 mg L^{-1}, followed by acetamiprid (828.59 mg L^{-1}), imidacloprid (2901.96 mg L^{-1}), lufenuron (4490.86 mg L^{-1}), flubendiamide (9452.28 mg L^{-1}), and emamectin benzoate (12,802.79 mg L^{-1}).

After 12 h, the LC_{50} value of chlorpyrifos further declined to 81.13 mg L^{-1}, followed by acetamiprid (358.66 mg L^{-1}), imidacloprid (390.32 mg L^{-1}), lufenuron (2304.24 mg L^{-1}), emamectin benzoate (3668.60 mg L^{-1}), and flubendiamide (5118.96 mg L^{-1}).

After 24 h, the LC_{50} value of chlorpyrifos was 44.85 mg L^{-1}, followed by acetamiprid (218.04 mg L^{-1}), imidacloprid (253.67 mg L^{-1}), lufenuron (1452.11 mg L^{-1}), emamectin benzoate (1140.86 mg L^{-1}), and flubendiamide (4215.51 mg L^{-1}). After 48 h, the LC_{50} value of chlorpyrifos further reduced to 34.99 mg L^{-1}, followed by acetamiprid (31.75 mg L^{-1}), imidacloprid (223.95 mg L^{-1}), lufenuron (877.87 mg L^{-1}), emamectin benzoate (580.28 mg L^{-1}), and flubendiamide (2325.36 mg L^{-1}).

Mean percentage mortality

The mean percentage mortality of *B. hebetor* and the comparison of all tested insecticides demonstrated their relative toxicity levels. Chlorpyrifos exhibited the highest mortality rates, with 48.00%, 57.33%, 66.66%, 76.00%, and 88.00% recorded after 3, 6, 12, 24, and 48 h, respectively. The highest overall mortality (88%) was observed with Chlorpyrifos against *B. hebetor*, followed by Acetamiprid (72.00%), Imidacloprid (68.00%), Lufenuron (64.66%), Emamectin Benzoate (52.00%), and Flubendiamide (40.00%) after 48 h (Table 3).

DISCUSSION

The present study demonstrated the toxic effects of six synthetic insecticides on *B. hebetor*. High LC_{50} values indicated low toxicity, whereas low LC_{50} values signified high toxicity. The results revealed that chlorpyrifos had the lowest LC_{50} value, making it the most toxic insecticide against *B. hebetor*, followed by acetamiprid, imidacloprid, lufenuron, emamectin benzoate, and flubendiamide. Acetamiprid and imidacloprid exhibited high toxicity, lufenuron and emamectin benzoate showed moderate toxicity, while flubendiamide had the lowest toxicity against the larval parasitoid.

The findings of this study are consistent with those of Muslim et al. (2018), who reported that chlorpyrifos exhibited high toxicity against *B. hebetor* due to its low LC_{50} value, followed by Patra and Samanta (2017). Mahdavi et al. (2015) also found that chlorpyrifos was highly toxic to *B. hebetor*, with the lowest LC_{50} value of 3.57 ppm. Similarly, Faal-Muhammad-Ali et al. (2014) observed that chlorpyrifos adversely affected both immature and adult stages of the parasitoid. Moreover, Lorsban (chlorpyrifos 40% EC) demonstrated significant toxicity, causing maximum mortality of *B. hebetor* within 6 to 24 h post-treatment.

Furthermore, Khan et al. (2009) identified chlorpyrifos as the most toxic insecticide against *B. hebetor*. The present study also confirms that Lorsban (chlorpyrifos) was highly toxic to the larval parasitoid at both lethal and sublethal doses. Overall, these findings align with the results of previous studies.

According to Shankarganesh et al. (2017), imidacloprid was recorded as a moderately to highly toxic insecticide against larval parasitoids. Rafiee-Dastjerdi et al. (2012) reported that imidacloprid had an adverse effect on *B. hebetor*, causing mortality at high concentrations. Similarly, Preetha et al. (2010) found that imidacloprid exhibited high toxicity, resulting in 70% mortality of *B. hebetor* within 48 h post-treatment. This aligns with our findings, which also indicate significant toxicity of imidacloprid; however, the degree of

impact may vary depending on factors such as exposure time, concentration, and environmental conditions. Sarmadi et al. (2010) reported that imidacloprid had a moderately toxic effect on all studied biological parameters and negatively impacted the gross reproductive rate of *B. hebetor*. These results are consistent with previous studies, confirming that imidacloprid contributes to the mortality of *B. hebetor*.

Table 2. LC_{50} values of different insecticides against *B. hebetor* at specific time intervals.

Insecticide	Time (h)	LC_{50} (mgL^{-1})	Slope ± SE	Chi square	95% FL (upper-lower)	P	Df
Acetamiprid	3	1397.09	0.79±0.21	0.12	712.06-8369.88	0.92	5
	6	828.59	0.58 ± 0.19	0.26	413.97-8693.29	0.48	5
	12	358.66	0.57±0.19	0.36	171.18-1243.03	0.88	5
	24	218.04	0.62± 0.19	0.25	89.31- 441.19	0.46	5
	48	31.75	0.53± 0.20	0.26	0.06-90.47	0.55	5
Imidacloprid	3	16770.81	0.71±0.19	0.24	9238.89-77221.75	0.84	5
	6	2901.96	0.38±0.18	0.14	0.03-8312.49	0.54	5
	12	390.32	0.44±0.19	0.02	121.00-1492.70	0.78	5
	24	253.67	0.45±0.20	0.09	0.000-1067.76	0.57	5
	48	223.95	0.53±0.21	0.08	0.00-1026.07	0.92	5
Lufenuron	3	19490.57	0.71±0.19	0.19	8683.48-96726.87	0.97	5
	6	4490.86	0.43±0.18	0.13	728.54-16535.47	0.49	5
	12	2304.24	0.62±0.196	0.48	475.31-4321.76	0.99	5
	24	1452.11	0.46±0.19	0.13	340.02-3488.50	0.78	5
	48	877.87	0.55± 0.20	0.21	10.28-2193.28	0.95	5
Flubendiamide	3	52893.76	0.52±0.19	0.26	17213.94-48107768.00	0.99	5
	6	9452.28	0.40±0.18	0.28	3726.18-9135633.00	0.92	5
	12	5118.96	0.53±0.193	1.09	1821.67-14318.02	0.29	5
	24	4215.51	0.64±0.19	0.85	1758.65-8211.15	0.96	5
	48	2325.36	0.73±0.19	2.26	761.15-3990.71	0.79	5
Emamectin benzoate	3	54089.84	0.53±0.20	0.85	17613.12-39847836.00	0.67	5
	6	12802.79	0.42±0.18	0.04	5467.40-8990839.00	0.79	5
	12	3668.60	0.45±0.189	0.15	1442.21-9503.47	0.72	5
	24	1140.86	0.33±0.19	0.10	1698.11-7890.45	0.64	5
	48	580.28	0.42±0.19	0.13	0.00-1951.33	0.67	5
Chlorpyrifos	3	1901.16	0.65±0.19	0.14	413.94-3440.91	0.78	5
	6	249.79	0.42±0.19	0.86	0.00-1169.04	0.64	5
	12	81.13	0.38±0.215	0.12	158.24-688.20	0.40	5
	24	44.85	0.46±0.22	0.04	0.00-472.51	0.62	5
	48	34.99	0.62±0.24	1.19	0.00-495.39	0.66	5

Table 3. Mean percent mortality of *B. hebetor* exposed to six synthetic insecticides.

Sr. No.	Insecticides	Mean percentage mortality ± Standard Error at				
		3	6 h	12 h	24 h	48 h
1	Acetamiprid	32.00B±2.30	44.00B±2.30	2.00B±2.30	61.33B±1.33	72.00B±2.30
2	Imidacloprid	26.66BC±1.33	34.66C±2.66	42.66C±1.33	53.33BC±3.52	68.00B±2.30
3	Lufenuron	21.33CD±1.33	29.33CD±1.33	37.33CD±1.33	48.00CD±2.30	64.66BC±2.90
4	Flubendiamide	9.33E±1.33	17.33E±1.33	25.33 E±1.33	30.66E±1.33	40.00D±2.30
5	Emamectin	17.33DE±1.33	24.00DE±00	30.66DE±1.33	40.00DE±2.30	52.00CD±4.61
6	Chlorpyrifos	48.00A±2.30	57.33A±1.33	66.66A ±1.33	76.00A±2.30	88.00A ±2.30

According to Khan et al. (2009), lufenuron was found to be a low to moderately toxic insecticide against *B. hebetor* after 48 h of application. Similarly, Hooshmandi et al. (2015) reported that lufenuron exhibited moderate toxicity during the initial hours of application. The findings of the present study align with those of Khan et al. (2009).

Khan et al. (2009) also reported that emamectin benzoate was a low-toxicity insecticide against *B. hebetor*. However, the present study indicates that while emamectin benzoate was initially less toxic, its toxicity slightly increased over time. These results are consistent with those of Khan et al. (2009).

According to Shankarganesh et al. (2017), acetamiprid exhibited moderate to high toxicity against the larval parasitoid after 48 h of treatment. The present findings confirm the observations of Shankarganesh et al. (2017).

Previous studies have shown that flubendiamide had minimal adverse effects on *B. hebetor*, suggesting that this compound is compatible with the larval parasitoid. In the present study, emamectin benzoate was found to be harmless during the initial hours of application but caused mortality in *B. hebetor* after 48 h of treatment.

This study focused on a specific set of insecticides, which may not fully represent the wide range of chemical compounds used in agricultural pest management. Future research should explore a broader spectrum of insecticides, particularly those with novel chemistries and lower non-target toxicity. Moreover, field trials would provide a more comprehensive understanding of insecticide compatibility with biological control programs.

CONCLUSION

The results of the current study concluded that flubendiamide and emamectin benzoate are highly recommended as they are more eco-friendly and may be used in IPM programs for the management of lepidopteran pests along with the biocontrol agent *B. hebetor*. Lufenuron is moderately toxic, whereas acetamiprid and imidacloprid are more toxic, and chlorpyrifos exhibited high toxicity against *B. hebetor*. Future research should focus on field-based evaluations to validate these laboratory findings, assess sub-lethal effects on the behavior of *B. hebetor* and reproduction, and explore alternative biopesticides that align with sustainable pest management goals.

AUTHORS' CONTRIBUTIONS

SA conducted the experiments and collected the data; DH and MA conceived and supervised the study; KZ and SM analyzed the results and US proofread the manuscript.

CONFLICT OF INTERES

The authors declare no conflict of interest.

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